mean-field theory with the PB equation, which under low field condition be further simplified to the linearized Poisson-Boltzmann equation (LPBE). A variety of analytical and numerical techniques have been developed to solve the PB equation. Our group has achieved a fundamental result in deriving the first completely general analytical solution to the linearized Poisson Boltzmann equation (LPBE) for computing the screened (salty) electrostatic interaction between arbitrary numbers of nanoscale spheres of arbitrarily complex charge distributions, separated by arbitrary distance (or concentration), adapted from the generalized Kirkwood PBE solution. This analytical solution in turn serves as the foundation of a semi-analytical approach to solving the LPBE for any nanoscale shape (PB-SAM). We have recently incorporated PB-SAM into a Brownian Dynamic approach to create a robust coarse-grained simulation tool to study a range of biological systems, including a recent study of Barnase/Barstar association under crowded conditions. We have developed a highly parallelized version of PB-SAM that is competitive with current PBE software, and are exploring 3-body implementations to further increase PB-SAM efficiency.

2083-Pos Board B813

Simple Method for Hybrid All-Atom and Coarse-Grained Molecular Dynamics Simulations and Its Applications

Sun Mi Choi[1,2], Pandian Sokkar[2], Young Min Rhee[1,2].
[1]POSTECH, Pohang, Republic of Korea, [2]Center for Self-assembly and Complexity, Institute for Basic Science, Pohang, Republic of Korea.

Hybrid all-atom (AA) and coarse-grained (CG) simulation has the possibility of overcoming the limitations of both AA and CG molecular dynamics (MD) simulations. Hybrid AA/CG systems are practicable to simulate microsecond time scale and to get detailed information for molecular structures. Many existing methods for hybrid AA/CG simulations tend to require heavy parameterizations that complicate multiscale simulations. We test a simple scheme for simulating hybrid AA/CG systems using standard AA and CG force fields, together with four small proteins as test cases for the scheme. Our method uses virtual sites for interactions between CG and AA resolution as reported earlier (Rzepiela et al. Phys. Chem. Chem. Phys. 2011, 13, 10437-10448) and we add distance restrained FG water layer to improve the reliability of the hybrid simulation. We observe that the addition of distance restrained FG water layer in the hybrid simulation results in close accord with the structure from the atomistic simulations. However, there are also digressions from correct behaviors that need to be addressed in future developments. We show such various results in detail and discuss the prospects of our scheme.

Biosensors I

2084-Pos Board B814

Nanopore Quantitation of Cancer BRAF Driver Mutation Facilitated by a DNA Interstrand Merculock

Kai Tian, Xi Fang, Corbin Reagan, **Li-Qun Gu**.
Bioengineering, University of Missouri, Columbia, MO, USA.

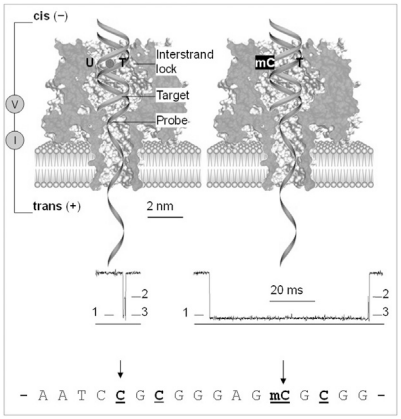
Driver mutations are a special type of genetic alterations that are causally correlated with oncogenesis. Accurate detection of the presence of driver mutations is extremely useful for early cancer diagnosis. BRAF Serine/threonine-protein kinase B-raf) has a predominant driver mutation V600E, which occurs with the highest incidence mainly in melanoma, colorectal and thyroid cancers, and in other cancers. Currently the BRAF pathway has become a drug arget for molecular therapy. Here we devise a novel nanopore single-molecule assay to accurately detect this driver mutation. The BRAF V600E gene involves a single-nucleotide transversion T1799A in the sense strand, and A1799T in the antisense strand. We selected the antisense strand as the target. Upon hybridization with an optimized probe that contains a thymine at the mutation site, the target•probe complex can form a T-T mismatch. The nanopore single-molecule sensor can be used to visually discriminate this T-T mismatch bound with a mercury ion (Hg^{2+}). This is because the Hg^{2+} binding creates a reversible interstrand lock, called MercuLock, which enhances the hybridization strength by two orders of magnitude. Such MercuLock cannot be formed in A-T base pair between the normal BRAF gene and the same probe, suggesting that the MercuLock acts as a fingerprint of the mutant DNA. By counting the frequency of MercuLock blocking events in the nanopore, we can quantize trace amount of mutant DNA in the mixture. This approach can be adapted to the detection of any thymine-involved driver mutations and single nucleotide polymorphisms (SNPs) for cancer detection.

2085-Pos Board B815

Constructing CPG Site-Specific Interstrand Locks for Single-Molecule Epigenetic Detection in a Nanopore

Insoon Kang, Yong Wang, **Li-Qun Gu**, Corbin Reagan.
Bioengineering and Dalton Cardiovascular Research Center, University of Missouri, Columbia, MO, USA.

DNA methylation is an important epigenetic regulation of gene transcription. Locus-specific DNA methylation can be used as biomarkers in various diseases including cancer. Most current methods are for genome-wide methylation analysis, but clinical diagnostics demands simple, low cost and quantitative approach for determining methylation states at individual CpG sites in a gene fragment. The nanopore is providing an excellent single-molecule platform for genetic and epigenetic exploration. Using the nanopore single-molecule sensor, we identified that divalent Mercury ion (Hg^{2+}) can selectively bind a single uracil-thymine mismatch (U-T) in a dsDNA. The Hg^{2+} binding creates a reversible interstrand lock, called MercuLock, which enhances the hybridization strength by two orders of magnitude. Such MercuLock cannot be formed in a 5-methylcytosine-thymine mismatch (mC-T). By nanopore detection of dsDNA stability, single bases of uracil 5-methylcytosine can be distinguished. Since uracil is converted from cytosine by bisulfite treatment, cytosine and 5'-methylcytosine can be discriminated. We have demonstrated multiple CpG methylation analysis of a CpG island in the cancer-derived p16 gene. This single-molecule assay has potential in detection of epigenetic cancer biomarkers in biofluids, with an ultimate goal for early diagnosis of cancer.

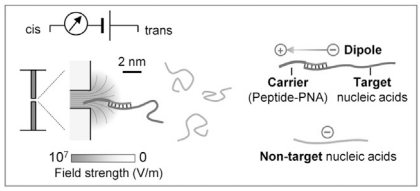


2086-Pos Board B816

Novel Nanopore Dielectrophoresis Mechanism for Selective Microrna Detection in Clinical Set

Kai Tian, Brandon Fricke, **Li-Qun Gu**.
Bioengineering, University of Missouri, Columbia, MO, USA.

The nanopore can electrophoretically trap single DNA/RNA molecules for genetic/epigenetic detections. However, low selectivity for complex samples (extracts from plasma) remains the challenge to clinical applications. We report an novel biophysical mechanism–***Carrier-guided-Nanopore-Dielectrophoresis***(CND)–for selective nucleic acids detection. We invented a polycationic micro-carrier. Upon hybridization with the target, the target•carrier forms a moment-tunable dipole, which can be attracted into the nanopore by dielectrophoresis from a huge electric field gradient (10^7V/m-per-nm) outside the nanopore entrance. In contrast, any non-target species without carrier hybridization carries negative charge and would electrophoretically migrate away from the nanopore. Consequently only the target•carrier nanopore signatures can be identified; any interference signal from non-target nucleic acids is completely eliminated. Unlike electrophoresis that lacks selectivity, the nanopore dielectrophoresis can selectively drive target nucleic acids of any size by using a universal micro-carrier. This represents the first and substantial step in translating the nanopore-sensor into a clinically-usable tool for molecular diagnostics. We demonstrate how to utilize this mechanism to accurately quantify cancer-derived microRNA biomarkers in patient plasma. *Nat.Nanotech.*_-DOI:10.1038/NNANO.2011.147. *ACS_Nano*_DOI:10.1021/nn305789z



2087-Pos Board B817

Detection of Single Biopolymers at High Current Bandwidth with Hafnium Oxide Nanopores

Joseph W. Larkin[1], Robert Henley[1], David C. Bell[2], Tzahi Cohen-Karni[3], Jacob K. Rosenstein[4], Meni Wanunu[1].
[1]Physics, Northeastern University, Boston, MA, USA, [2]School of Engineering and Applied Science, Harvard University, Cambridge, MA, USA, [3]Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology, Cambridge, MA, USA, [4]School of Engineering, Brown University, Providence, RI, USA.

Hafnium oxide (HfO2) is a chemically and mechanically stable insulator that may be deposited via atomic layer deposition. These properties make it an ideal material for ultrathin, free-standing membranes. We fabricate these membranes by depositing HfO2 on a low stress silicon nitride (SiN) film, and then locally removing the SiN. We then fabricate a nanopore in this HfO2 membrane with a 200 kV transmission electron beam. Coupled with a megahertz-bandwidth

current detection platform, nanopores in these <10 nm-thick HfO2 membranes can detect a wide variety of single biopolymers, including single-stranded DNA at a current blockage level comparable to that of the alpha-hemolysin biological nanopore. These HfO2 pores may be used for hours at a time and translocate tens of thousands of molecules without significant expansion. Interaction with the pore walls results in slow translocation times for nucleic acids. At the same time the thinness of the membrane increases the pore's electric field and confines the region of strong analyte-pore interaction to prevent clogging. These results indicate that HfO2 may be a superior material to SiN for single molecule nanopore experiments.

2088-Pos Board B818

Solid-State Nanopore Mapping of DNA with Site-Specific Bound Ligands

Autumn Carlsen[1], Osama K. Zahid[1], Jan Ruzicka[2], Ethan W. Taylor[2], Adam R. Hall[1].
[1]Virginia Tech-Wake Forest School of Biomedical Engineering and Sciences, Wake Forest University School of Medicine, Winston Salem, NC, USA, [2]Joint School of Nanoscience and Nanoengineering, University of North Carolina Greensboro, Greensboro, NC, USA.

We report the use of solid-state (SS-) nanopores to detect discrete ligands bound to specific sites along the length of double-stranded DNA. Binding to DNA templates is studied initially with electromobility shift assays and atomic force microscopy before SS-nanopore translocation. We find that DNA containing as few as 1-2 bound ligands can be resolved, demonstrating the feasibility of mapping tagged regions of genomic DNA at the single-molecule level.

2089-Pos Board B819

Hardware Implementation of Denoising Algorithms for Nanopore Sensing

Brett W. Larsen, Michael Goryll, Prasanna Sattigeri.
Arizona State University, Chandler, AZ, USA.

Effective biosensors continue to be a research area of great interest for both defense and medical applications. In particular, silicon pores with diameters in the range of micro/nano-meters have demonstrated the ability to detect an array of analytes. Typically, these sensors make use of the Coulter counter set-up where a drop in current across the chamber is observed when a biomaterial passes. The duration and amplitude of this drop is indicative of the biomaterial's size and shape.

In order to effectively use such sensors, however, robust denoising and classification algorithms must also be developed. Recently, Non-Positive Go Decomposition (NpGoDec) was shown to be an effective denoising method for biological data, correctly classifying simulated controlled data for Immunoglobulin G biomolecule with 96 percent accuracy.

In this work, a research team for Arizona State University programmed the NpGoDec algorithm onto a Field Programmable Gate Array (FPGA) for on-chip, biosensor processing. There are several benefits to such a system. First, denoising the signal on an FPGA reduces processing time by avoiding the transmission of the raw data into off-line processing software such as MATLAB and brings biological sensing one step closer to real time. In addition, performing much of the signal processing work on the FPGA moves the sensor closer to being a portable device. The system is carefully investigated for accuracy and processing time as compared to the original, simulated signal. Our approach also enables the integration of a classifier onto an FPGA, which will allow the system to quickly identify the biomaterials passing through a nanopore.

2090-Pos Board B820

Graphene Nanopore with Self-Aligned Plasmonic Optical Antenna

SungWoo Nam[1], Inhee Choi[2], Chi-cheng Fu[2], Kwanpyo Kim[3], SoonGweon Hong[2], Yeonho Choi[4], Alex Zettl[3], Luke P. Lee[2].
[1]Mechanical Science and Engineering, University of Illinois, Urbana-Champaign, Urbana, IL, USA, [2]Bioengineering, University of California, Berkeley, Berkeley, CA, USA, [3]Physics, University of California, Berkeley, Berkeley, CA, USA, [4]Biomedical Engineering, Korea University, Seoul, Republic of Korea.

The atomically thin nature of graphene makes it an ideal translocation membrane for high resolution, high throughput, single-molecule DNA sequencing based on nanopores. The conventional approach to creating nanopores on graphene requires a high-resolution electron beam sculpting/drilling process, which often suffers from process variability, precluding the platform from being scalable. Here, we report the formation of integrated graphene nanopores with self-aligned plasmonic optical antennae by photothermal sculpting. We show that a nanometer-sized heated spot created by photon-to-heat conversion (i.e., photothermal effect) of a gold nanorod resting on a graphene membrane forms a nanoscale pore with a self-integrated optical nanoantenna in a single step. The unique interface of graphene nanopore-plasmonic optical antenna is composed of a nanopore with a smallest achievable dimension of a few nanometers and a hemispherical gold nanoparticle located adjacent to the nanopore. The distinct plasmonic traits of metal nanoparticles, which concentrate micron-sized light into nanoscale regions, yield the significant advantage of parallel nanopore fabrication compared to the conventional sequential process using an electron beam. In addition, we achieve tunability of both the nanopore dimensions and the optical characteristics of plasmonic nanoantennae by controlling laser fluence and the dimension of nanoparticles. Finally, the optical function of our self-aligned plasmonic nanoantenna on graphene nanopore is manifested by multifold fluorescent signal enhancement during single lambda phage DNA translocation through a graphene nanopore. We believe our approach to forming an integrated graphene nanopore with self-aligned optical antenna could potentially offer a new avenue and advances for nanopore-based simultaneous electrical and optical DNA sequencing.

2091-Pos Board B821

Ion Conductivity, Structural Dynamics and the Effective Force in DNA Origami Nanopores

Chen-Yu Li[1], Jejoong Yoo[2], Aleksei Aksimentiev[3].
[1]Center of Biophysics and Computational Biology, University of Illinois at Urbana-Champaign, Urbana, IL, USA, [2]Center for the Physics of Living Cells, University of Illinois at Urbana-Champaign, Urbana, IL, USA, [3]Department of Physics, University of Illinois at Urbana-Champaign, Urbana, IL, USA.

Nanopores have emerged as convenient tools for single molecule manipulation and analysis. In a typical measurement, a charged biomolecule-DNA or a protein-is transported through a narrow pore in a biological or synthetic membrane by external electric field. The presence and, in some cases, the chemical structure of the biomolecules is detected by measuring changes in the ionic current that flows through the nanopore. Recently, it has become possible to combine solid-state nanopores with self-assembled DNA nanostructures, the so-called DNA origami, into hybrid pores of advanced functionality. In such systems, a DNA origami plate partially covers the solid-state nanopore, providing both a nanopore of well-defined chemical structure and a platform for incorporation of auxiliary systems such as processive molecular motors and/or metallic nanoparticles. Here, we report molecular dynamics simulations of DNA origami nanopores that characterized the microscopic properties of such systems with unprecedented resolution. First, we built accurate all-atom models of DNA origami nanopores based on the honeycomb and square lattices and simulated the models using the molecular dynamics method. Next, we determined the ionic conductivity of different DNA origami designs by performing the simulations under applied electric field. For some square lattice designs, we observed reversible changes in the DNA origami structures responsive to the magnitude of the applied electric field. In the final set of simulations, we studied the electrophoretic transport of double-stranded DNA through DNA origami nanopores and characterized the effective force exerted by the applied field on both the permeating DNA molecule and the DNA origami structure. Our simulations demonstrate the utility of the molecular dynamics method for rational engineering of DNA origami nanopores into nanoscale sensors of advanced detection functionality.

2092-Pos Board B822

Tailoring Nanoprobes for Single-Cell Surgery

Paolo Actis[1], Sergiy Tokar[1], David Klenerman[2], Yuri Korchev[1].
[1]Imperial College London, London, United Kingdom, [2]University of Cambridge, Cambridge, United Kingdom.

Physiological and pathological processes within the human body are controlled by complex cell-cell interactions within the context of a dynamic microenvironment. Current methods are inadequate to monitor the multiple interactions and dissect the contributions of single cells to these processes. We will present the development of nanoprobes based on nanopipettes for manipulating living cells. We will describe the integration of these nanoprobes with scanning probe microscopy techniques to allow the delivery of biomolecules to individual cells and to biopsy minute amounts of cytoplasmic material and organelles from within living cells. In particular, we will discuss recent developments regarding the fabrication of carbon nanoelectrodes whose size can be precisely tuned with nanometer precision. Nanoelectrodes as small as 3 nm in radius can be functionalized with platinum using established electrochemical methods. The electrochemical deposition of platinum only slightly increases the surface area of the nanoelectrode but dramatically enhances its catalytic activity toward oxygen reduction and hydrogen peroxide oxidation. We will discuss their application for measurement of metabolic activity inside brain slices. Furthermore, the nanoelectrode can be precisely inserted into an individual neuron within the brain tissue with minimal disturbance to the biological milieu. We will present data showing the ability of the functionalized nanoelectrodes to measure intracellular endogenous molecules. Current studies in our group are trying to link the measured current with pathogenic conditions.